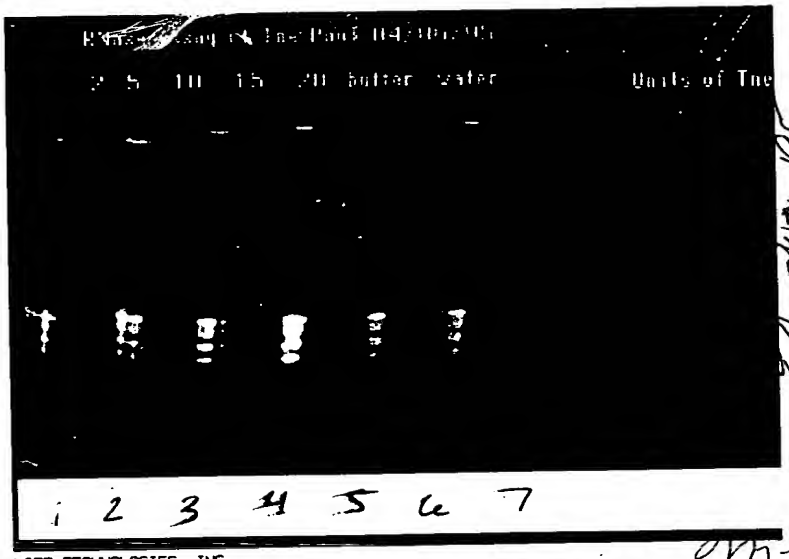
Project No._____
122 Book No._____ TITLE Completion of RNase Assay -

From Page No.__

Take samples from -20°c freezer - spin in micro centrifuge 15 minutes - decant etoh - air dry pellets - Add - ul of RNA blue juice - heat 30 m sec at 90°c Run out on 6% polyacrylamide sequencing gel - 400 volts -



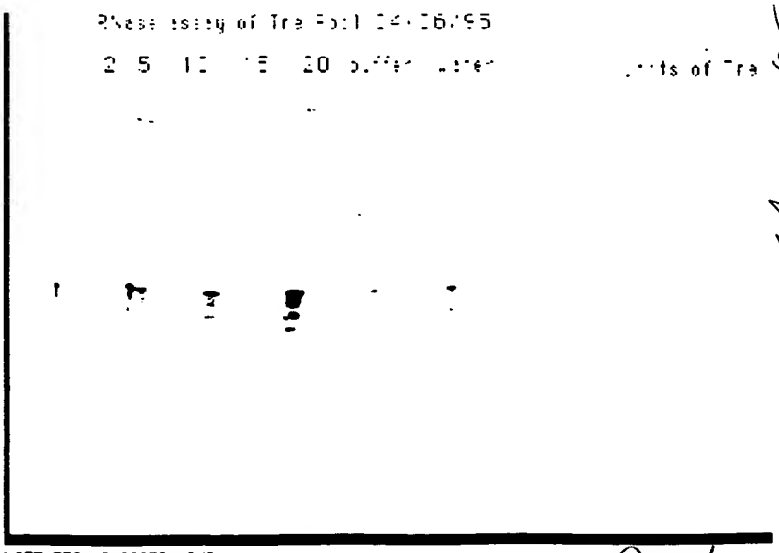
GT 04/04/95

MF 4/13/95

Conclusion - Appears to be RNase free! Next time use more RNA - Only used half of recommended amount Used 1ug vs recommended 2ug.

~~Bradford on Pads~~

GT 04/04/95



GT 04/04/95

MF 4/13/95

To Page N

Witnessed & Understood by me,	Date	Invented by E. Flynn	Dat
Mary Longo	4/13/95	R corded by	04/04/95

Exonuclease Assay - Tne Pool

Pag N .__

E. No. 30042 SOP -

Tube	Rxn Mix 45	Enzyme Units	µl	H_2O
1		~~0~~ 0	—	5 µl
2		~~10~~ 2.0	←	4 µl .5U/µl
3		~~20~~ 5	1	
4		10	2	
5		15	3	
6		~~30~~ 20	4	
7		0		5 µl dil'n buffer

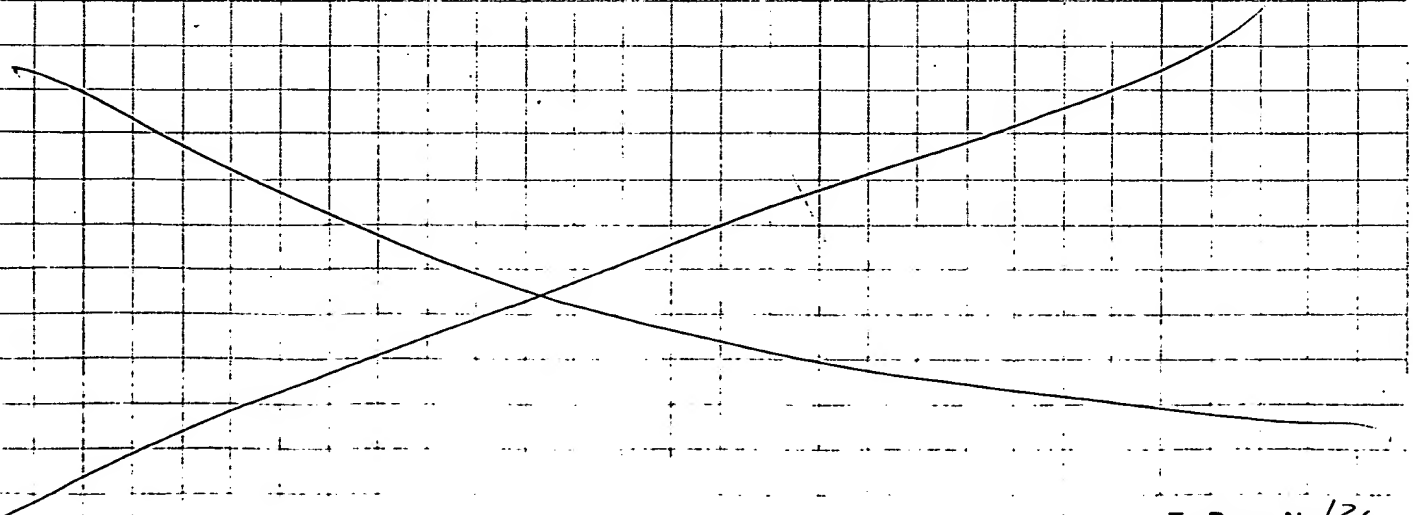
Rxn Mix

10x PCR	80
50mM $MgCl_2$	80
5' ds sub	16 pmol 32 µl .5 pmol/µl
3' ds sub	16 pmol 32 µl .5 pmol/µl
H_2O	404
	720

16 rxns -

Heat @ 37°C for 1 hour - 1-7
Heat @ 72°C for 1 hour - 8-14

see page - 126 for data



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ssed & Underst od by me,	Dat	Inv nt d by E. Hynes	Date
Mary Longo	4/13/95	Recorded by	04/[illegible]/95

[redacted]

Project No. _____
124 Book No. _____ TITLE Endo Assay - 18038 QCP_T

From Page No. _____

Rxn Mixture - fn 8 rxns - (all tubes on ice before use -)

	10x DCR buffer	40 µl ✓
	50mM $MgCl_2$	40 µl ✓
	ΦX174 (+) DNA -	8 µg (23.5 µl) ✓
15264-03	Autoclaved H_2O	256.5
.34 µg/µl		360 µl

	Endo Mix	H_2O
1	45	5
2	45	1
3	45	4
4	45	3
5	45	2
6	45	1
7	45	5 Dili Buffer ✓

Diluted enzyme 50/µl

2 units	4 µl
5 units	1 µl
10 units	2 µl
15 units	3 µl
20 units	4 µl

Incubate @ ~~72°C~~ 37°C for ~~3~~ 5.5 hours -

Tag

Double Stranded Assay -

	10x PCR buffer	40 ✓
	50mM $MgCl_2$	40 ✓
25260-027	ΦX174 RF	8 ~~24.2~~ 24.2 ✓
EF ~~RE~~ 1702	Autoclaved H_2O	259.8
.33 µg/µl		360 -

	Endo	H_2O
1	45	5
2	45	1
3	45	4
4	45	3
5	45	2
6	45	1
7	45	5 Dil'n buffer ✓

Dil Enzyme 50/µl

2	4 µl of 50/µl
5	1 µl
10	2 µl
15	3 µl
20	4 µl
—	

T Pag

Witnessed & Understood by me,	Date	Invented by	Date
Mary Longo	4/13/95	Rec rded by	04-04

Endo Assay -

Project N ______
Book N ______

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Spin samples down add 5 μl of Blue Juice -
Run out on 1.2 % Agarose gel -

1 2 3 4 5 6 7 8 9 10 11 12 13 14

SSingle Endo   DS- Endo

H_2O

1	2	3	4	5	6	7	~~8~~	~~9~~	~~10~~
H_2O	2	5	10	15	20	B			

8	9	10	11	12	13	14
H_2O	2	5	10	15	20	B

C = ~~H_2O~~ 1/60 at 10 u - 4.5 / 1.0 / 4.5 = conv 5 H_2O 10 ...

on
1/13/95

Endo looks good - however DS Endo - shows conversion to linear but this is also present in the buffer only lane - could just be a contaiminant in the Dil'n buffer -

Dil'n Buffer used - from A.G. Flakers from the 4°C Deer cooler - orange tip -

Conclusion: - free of SS Endo nuclease - possible some DS endo nuclease but control with buffer only shows significant conversion to ~~form~~ linear so believe ~~that~~ the dil'n buffer ~~in~~ ~~ex~~ has DS endo ~~...~~ activity not the enzyme prep.

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...essed & Understood by m ,	**Date**	**Invented by** S. Flynn	**Date**
Mary Longo	4/13/95	**Recorded by**	05-04-95

[redacted]